Coffee Silverskin Extract for Aging and Chronic Diseases

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ABSTRACT

Coffee silverskin (CS) is the by-product generated when roasting coffee, which is very abundant worldwide. Its potential as a functional food ingredient was for the first time proposed at the beginning of 2000. However, to the best of our knowledge its use as a functional food ingredient is not commercially available. Initially, CS was proposed as a natural source of prebiotic or dietary fiber. Recently, novel information regarding the chemical composition and innovative applications of this particular food matrix and its derivatives has been reported. In the last decade the valorization of food wastes has become a priority research line in order to achieve a sustainable world and a better future for Earth. On the other hand, it is mandatory to find natural and sustainable strategies to either reduce the risk or treat chronic diseases, especially those related to demographic changes such aging of the population and those considered epidemics of the XXI century (obesity and diabetes). The present chapter provides some insights on the extraction process, chemical composition and health promoting properties of aqueous coffee silverskin extract (CSE). The health promoting properties of CSE are attributed to its powerful antioxidant character. Therefore, CSE can be used to prevent or treat age related chronic diseases caused by oxidation and inflammation. The chapter pretends to demonstrate how bioeconomy may be helpful to achieve an environmentally clean and healthy world; as well as, to pin-point the valorization of by-products into functional food ingredients as a good strategy for the future. In conclusion, the data presented in the chapter support that coffee can be a sustainable industry and a source of several multifunctional bioproducts of interest for human health.

Keywords: Coffee by-products; sustainability; aging; obesity; diabetes; coffee silverskin; chronic diseases; coffee antioxidants; oxidative stress; inflammation

INTRODUCTION

Food has a vital role in maintaining our health properly, and also helps in the prevention and cure of diseases. Nowadays, more than 95% of all chronic disease is caused by food choice, toxic food ingredients, nutritional deficiencies and lack of physical exercise. Food with good nutrients builds health, and unhealthy food leads to several diseases. Our eating habits have changed dramatically during the 20th century. In the last decades the market of functional foods has dramatically increased. Many plant extracts and natural compounds are emerging as functional candidates for the reduction of risk of non-communicable chronic diseases. Vegetable by-products are sustainable sources of bioactive compounds with health promoting and therapeutic properties to satisfy consumer's demands. The recycling of food wastes into health promoting products is of great interest worldwide, and it has great socioeconomic and environmental impact so far.

Coffee is one of the most frequently consumed drinks in the world. Total production in 2014/15 was estimated at 141.9 million 60 kg bags (International Coffee Organization, 2015). During processing to obtain the coffee beverage from roasted beans, over 90% of the coffee cherry is discarded as an agricultural by-product, which contains health promoting phytochemicals. As a consequence, the coffee industry is responsible for the generation of large amounts of wastes. This waste causes water pollution problems in rivers and insalubrious in the land areas near the cities or towns where it is discarded. The valorization of such wastes using the biorefinery approach represents a real contribution of many industries for sustainable and competitive development [1].

Coffee beverage is known for the antioxidant properties of its components such as caffeine, hydroxycinnamic acids including chlorogenic acid (CGA), and melanoidins [2]. Several epidemiological studies document the protective effect of coffee components in the risk of chronic diseases such as age related metabolic disorders associated to oxidative stress and inflammation including diabetes [3]. The present chapter focuses the attention on the potential of an extract from a coffee by-product named coffee silverskin (CS) obtained by application of simple and green technologies for preventing accelerated aging and age related chronic diseases associated to oxidative stress. Some insights on the extraction process, chemical composition and health promoting properties of aqueous coffee silverskin extract (CSE) are summarized in the following paragraphs.

Nature and chemical composition of coffee silverskin extract

CS is a thin tegument of the outer layer of the two beans, forming the green coffee seed and represents about 4.2 % (w/w)[4] (Figure 1 A). CS is the only by-product produced in the roasting process, and large amounts of CS are produced by large-scale coffee roasters in consuming countries (Figure 1 B).

Our research group patented an aqueous CSE from Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*) (WO 2013/004873) [5] (Figure 1 C). CSE is obtained using environmentally friendly technologies. The method of CSE preparation comprises no prior grinding step, spraying, or equivalent method to obtain coffee silverskin powder. Therefore, the process allows the obtaining of the extract defined in this application from whole coffee silverskin saving time,

energy, and simplifying the procedure. The extraction of phytochemicals and nutrients from the food matrix can take place with water at a temperature of about 100°C for at least 10 min (low technology). The extraction process can be also done under subcritical water conditions at a temperature of 50°C and a pressure of about 1500 psi, without prior grinding step, spraying or equivalent method (high technology). For long term storage the extracts obtained by the process described in the present patent application, can be frozen and lyophilized or spray-dried. The resulting powder (Figure 1 C) can be stored and protected from light, and frozen until use. The determination of the antioxidant capacity of the sample during storage at room and freezing temperatures show that under these conditions, the antioxidant properties are preserved for at least 6 months, and for more than two years when it is stored at -20ºC.

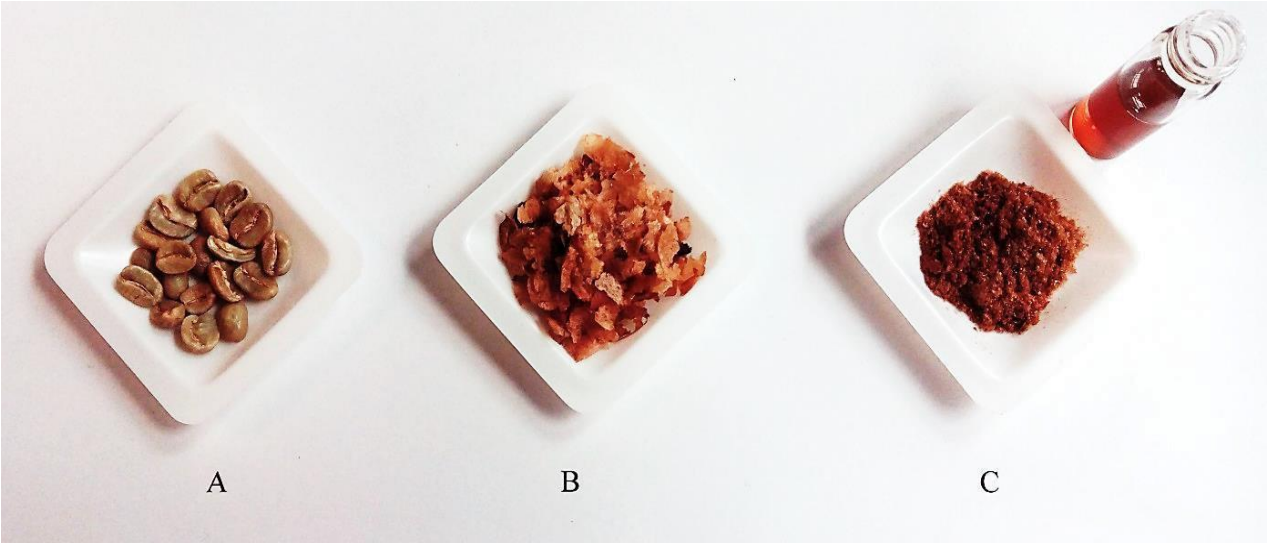


Figure 1. Green coffee beans in silverskin (A), coffee silverskin released during roasting of green coffee beans (B) and powdered coffee silverskin extract (WO 2013/004873) and in aqueous solution (10 mg/mL) (C).

Table 1 summarizes the chemical composition of the CSE. The patented CSEs are rich in total dietary fibre (28-36%), which includes about 4-9 % insoluble dietary fibre and 24-26 % soluble dietary fibre. CSEs are a good source of polyphenols, particularly CGA (1-6%); the most relevant are 5-O-, 3-O- and 4-O-caffeoylquinic acids [6]. CSE is also a good source of caffeine (3%), and melanoidins (17-23%) which are formed during the roasting process [6]. Coffee melanoidins are formed by polysaccharides, proteins and CGA, and exerted antioxidant capacity [7]. CSEs present a high proportion of extractable antioxidants in aqueous solution.

Antioxidant properties of coffee silverskin extract

CSE presents high antioxidant capacity equivalent to values > 0.85 g of CGA / 100g [8]. It has been suggested that the presence of CGA and melanoidins contributes to the antioxidant properties of CSEs *in vitro* [8–10]. Other coffee constituents are relevant for oxidative stress such as melatonin, lignans and lignin, tannic acid, isoflavones and trigonelline. All of these compounds may be present in CSE. Further investigation should be performed to gain insight on their individual contribution to the overall antioxidant effect of CSE *in vitro* and *in vivo*.

However, studies performed by our work suggest that those antioxidants with molecular weight < 10KDa are the principal contributors to the overall antioxidant power of CSE (data not shown). Since CGA is considered the major antioxidant of coffee, the role of this compound in the antioxidant properties of CSE has been studied.

Table 1. Chemical composition of coffee silverskin extracts.

Compounds	**ACSE (per 100g)**	**RCSE (per 100g)**
Proteins (g)	5.36	0.99
Carbohydrates (g)	5.44	13.43
Total dietary fibre (g)	28.69	36.21
Soluble dietary fibre (g)	24.01	26.80
Insoluble dietary fibre (g)	4.67	9.41
Caffeine (g)	3.02	3.39
Melanoidins (g)	17.26	23.94
CGAs (g)	1.12	6.85
Total phenolic content (g)	3.10	3.54
ORAC (mmol TEAC)	119.4	151.3
DPPH (mmol TEAC)	21.9	23.1
ABTS (mmol TEAC)	8.5	22.5
FRAP (mmol TEAC)	82.9	64.0

ACSE, Arabica coffee silverskin extract; RCSE, Robusta coffee silverskin extract; CGAs, chlorogenic acids; TEAC, Trolox

There are several studies that have investigated the antioxidant capacity of CGAs using different cell-based models. They concluded that this polyphenol protects against oxidative stress by diverse mechanisms: 1) alleviating DNA damage [11,12], 2) suppressing the mitochondrial membrane depolarization [13] and 3) improving the antioxidant defence in cells [14,15]. Other studies conducted in rodent models have confirmed the efficacy of dietary intake of CGA in preventing oxidative stress pathogenesis through increasing the level of non-enzymatic antioxidants (GSH and Vitamins C and E) and antioxidant enzymes (superoxido dismutase, catalase, GPx and glutathione-S-transferase) in diabetic model rats [16,17].

OXIDATIVE STRESS AND AGING

Aging is defined as an accumulation of changes in the cell, tissue, or an organ over time which leads to lose its function and vitality, undergoing mortality [18]. A major cause of aging is oxidative stress, which is defined as the imbalance between reactive oxygen species (ROS) and

antioxidants. Generally, cells are able to balance the production of oxidants and antioxidants. However, when cells are subjected to excessive levels of ROS or as a result of antioxidant depletion, oxidative stress occurs [19]. Reactive oxygen species include neutral molecules (hydrogen peroxide, H_2O_2), ions (superoxide anion, O_2^-) and radicals (hydroxyl radicals, $OH^{\cdot}$). Such molecules possess inherent chemical properties that confer reactivity to different biological targets [20].

Under normal conditions, ROS are natural byproducts produced in mitochondria, peroxisome, and plasma membrane which have positive physiological effects on cells, such as killing microorganisms, acting as a second messenger in cellular differentiation and proliferation and regulating signal transduction [21]. However, ROS can also be generated by exogenous sources (UV radiation or chemical agents) and cause DNA, protein, and lipid damage [22].

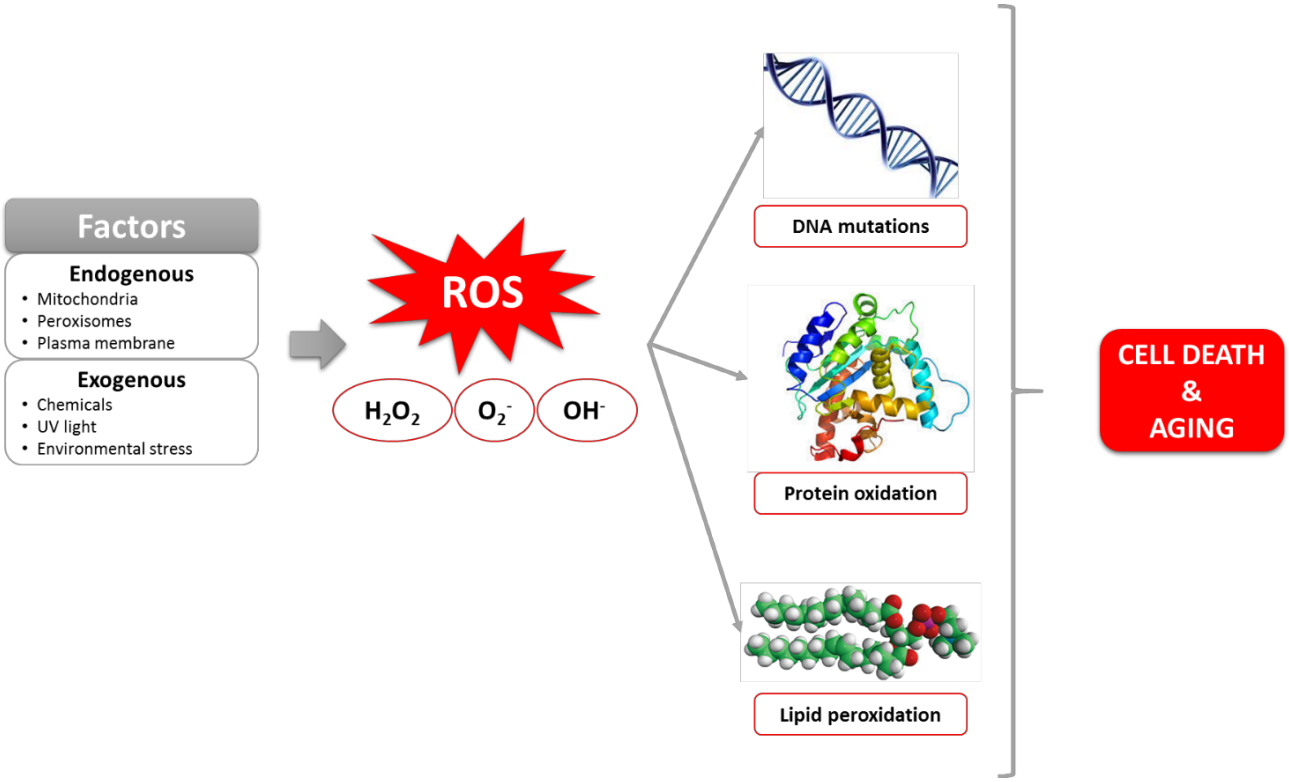
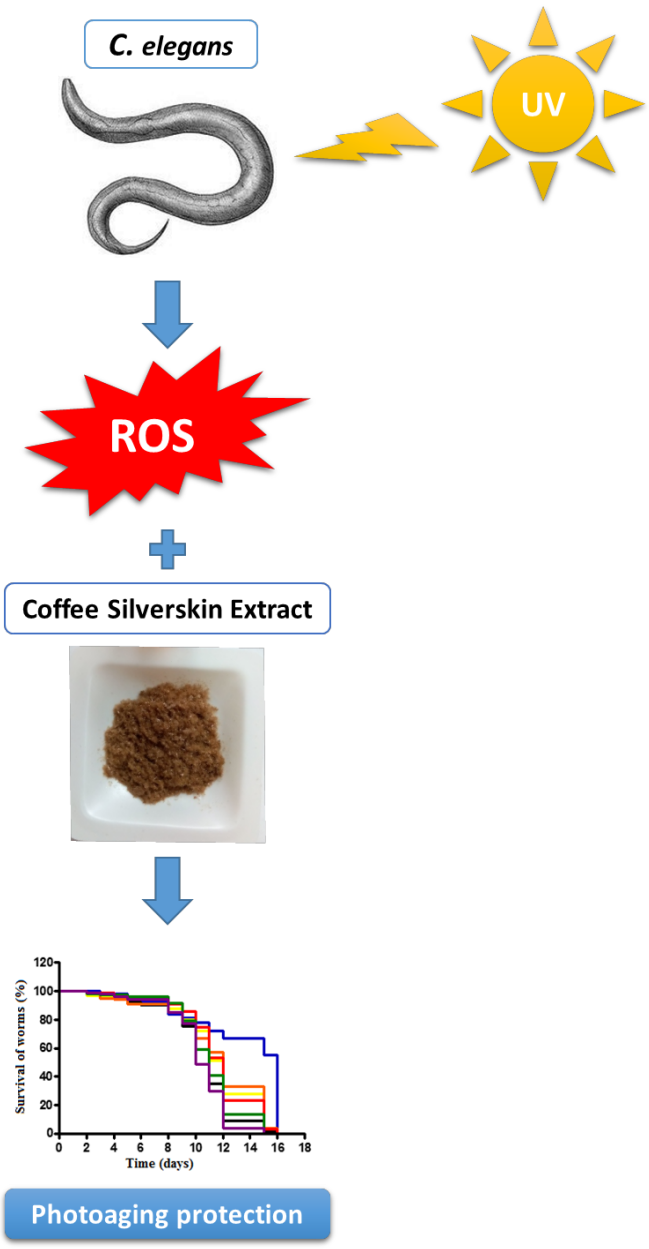


Figure 2. Molecular targets of oxidative stress.

The combination of DNA mutations, protein oxidation and lipid peroxidation induces a cellular progressive decline as a result of insufficient supply of energy leading to oxidative stress induced aging (Figure 2) [23]. In the process of aging, there is a deficiency of the endogenous antioxidant defenses of cells and the residual ROS generate oxidative stress, even in physiological conditions [18]. Exogenous sources of antioxidants such as a diet rich in fruits and vegetables (5 units/day) and the intake of natural supplements may be helpful to delay the aging process and age related chronic diseases. Large epidemiological studies support the relationship between oxidative state and global health; high consumption of foods rich in antioxidants is associated with lower disease rates and preventive protection [24]. The anti-aging effect of CSE has been investigated *in vivo* employing as an animal model *C. elegans* [25]. Results derived from this investigation are discussed in the present chapter.

Antiaging effect of coffee silverskin extract

C. elegans has been widely used in aging studies for two reasons: it is a multicellular organism with a fully sequenced genome, and it has a short lifespan. This nematode is also revealed to have evolutionarily conserved pathways for aging [26]. In this context, *C. elegans* is the ideal model since it combines topical and oral antioxidant administration, which is the favored recommendation [27]. Additionally, *C. elegans* is becoming a fast and inexpensive *in vivo* tool for screening the anti-aging effect of natural products such as CSE. There is no ethical problem in the use of *C. elegans*, as this nematode is not regarded as an animal in the EU regulation (Directive 2010/63/EU), and results obtained are consistent with higher animal models, which enable subsequent pre-clinical and clinical trials to be more oriented.



In this sense, preclinical data regarding the antiaging properties of CSE on *C. elegans* have recently been collected [25]. In this study, accelerated aging was induced by ultraviolet radiation C (UVC). The nematodes treated with CSE (1 mg/mL) showed a significant increased longevity compared to those cultured on a standard diet (Figure 3). The increased longevity observed was similar to that of the nematodes fed on CGA or vitamin C (0.1 µg/mL). The antiaging properties of the CSE observed in this study are due to its antioxidant character associated to phenols among other bioactive compounds present in the botanical material. Chlorogenic acids, caffeine, melanoidins and other bioactive compounds all together in the food matrix may act in a synergic manner when protecting from UV induced accelerated aging.

Figure 3. Effect of CSE (coffee silverskin extract) on induced oxidative damage of skin models.

There are other plant extracts containing CGA and other polyphenols able to exert an antiaging effect *on C. elegans.* For instance, crude blueberry extract and blueberry polyphenols (including an hydroxycinnamic ester fraction containing CGA) have lengthened the nematode's mean lifespan by 28% [28]. Moreover, Vayndorf et al. (2013) observed that when *C. elegans*

was pre-treated with whole apple extracts, worms were more resistant to stresses such as heat, UV radiation and pathogenic infection, suggesting that cellular defence and immune system functions were improved. The authors suggest a possible antioxidant mechanism underlying the antiaging effects of whole apple phytochemicals [29]. In addition, polydatin, a natural resveratrol glycoside, was found to significantly extend the mean lifespan of worms by up to 30.7% and 62.1% under normal, and heavy metal induced acute stress conditions, respectively [30]. Some of these extracts have already shown their effectiveness as antiaging agents in humans [31].

Oxidative stress, inflammation and age related chronic diseases

A relevant source of ROS is the inflammatory process, which induces oxidative stress and reduces cellular antioxidant capacity [32]. When the cellular immune system interacts with antigens, ROS are produced, and this leads to signaling cascades which trigger the production of proinflammatory cytokines and chemokines [33]. Inflammation is closely related to such chronic diseases: diabetes, obesity and the metabolic syndrome [34]. The main molecular links between inflammation and obesity are: 1) Inflammatory cytokines. TNF-α is overproduced in the adipose as well as muscle tissues of obese humans and also contributes to insulin resistance [35]. 2) Molecules produced in adipocytes such as leptin. This hormone plays important roles in immunity, and both mice and humans lacking leptin function exhibit impaired immunity [36]. 3) Lipids. Hyperlipidemia in obesity is responsible in part for inducing peripheral tissue insulin resistance [37].

On the other hand, both oxidative stress and inflammation are intimately linked with the development of diabetes. An increase in oxidative stress-derived inflammation is a major mechanism in the pathogenesis of diabetic nephropathy. Furthermore, an increase in inflammatory cytokine levels in diabetes may lead to a further increase in oxidative stress, as renal injury becomes more pronounced setting up a vicious cycle (Figure 4) [38].

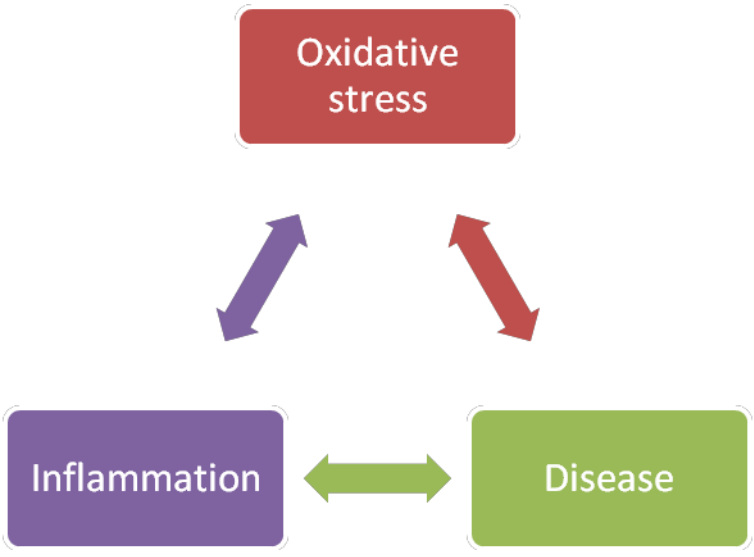


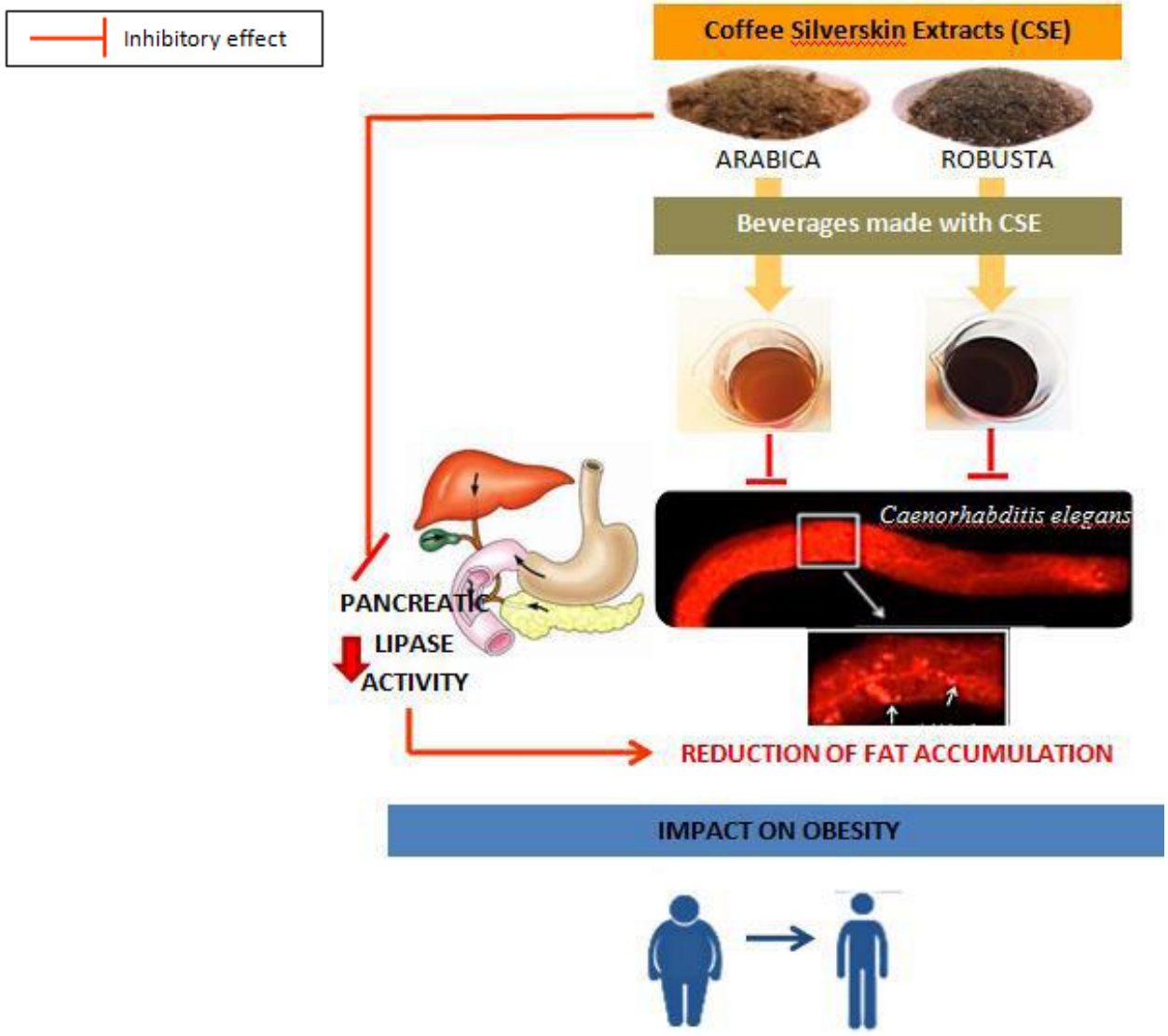
Figure 4. Oxidative stress and inflammation work together creating a vicious cycle.

Recently, the impact of the CSE in obesity and diabetes has been evaluated. Relevant information for supporting the biological impact of the CSE on the pathogenesis is discussed in the present chapter.

COFFEE SILVERSKIN EXTRACT AND OBESITY

Overweight and obesity are emerging as a major cause of the metabolic syndrome, which is increasing rapidly in modern societies [39]. According to the World Health Organization (WHO), obesity is defined as abnormal or excessive fat accumulation in subcutaneous tissues and the abdominal cavity that may impair health. The abdominal obesity correlates with the occurrence of diabetes mellitus, increasing the morbidity risk by 40× and 60.9× in individuals with a body mass index of 35 and >35 kg/m2, respectively, and worsening diabetes in 80% of obese diabetics [40].

Figure 5. Inhibitory effect of coffee silverskin extract (CSE) on fat accumulation and lipase activity.



Treatment should be aimed at weight loss by increased exercise and improved dietary habits, with a reduction in total calorie intake and reduced saturated fatty acid intake. Medical therapy can be initiated if lifestyle changes are insufficient. Search for novel foods or supplements to prevent and treat obesity is a priority worldwide. Plant extracts have attracted much attention as potential therapeutic agents in the prevention and treatment of obesity to their multiple targets and less toxic side effects [41].

The inhibitory effect of antioxidant novel beverages based on CSE from Arabica and Robusta coffees on fat accumulation has been studied *in vivo* by employing as animal model *C. elegans* [9]. A significant dose-response effect on reducing accumulation of body fat was found for pure CGA (3.54 mg/L) and caffeine (4.85 mg/L), achieving 30% and 29% reduction of lipid deposits, respectively. As was expected, the brews of Arabica and Robusta CSE (100 µg/mL), which contained physiologically active doses of these compounds, indicated to be effective by causing a body fat reduction of 21% and 24%, respectively. Furthermore, similar findings were described for body fat reduction from both Robusta CSE beverages and a commercial dietary supplement, made from Robusta decaffeinated green coffee extract. Therefore, a new beverage made from roasted CSE was obtained as natural alternative to dietary supplements for the prevention of overweight and obesity (Figure 5).

Dyslipidemia is a multifactorial disorder observed in obesity that includes hepatic overproduction of very low density lipoproteins, decreased circulating triglycerides lipolysis and impaired peripheral free fatty acid (FFA) trapping, increased FFA fluxes from adipocytes to the liver and other tissues and the formation of small dense low density protein (LDL) [42]. These metabolic disorders increase the risk of development of type 2 diabetes (T2D) and cardiovascular diseases and contribute to high rates of mortality and morbidity worldwide [43].

Orlistat (tetrahydrolipstatin) was approved as a prescription product by FDA in 1999 for obesity management in conjunction with a reduced caloric diet, and to reduce the risk of regaining weight after prior weight loss (http://www.fda.gov/). This drug is a pancreatic and gastric lipase inhibitor whose primary effect is to reduce fat uptake by the gut [44]. This is one of few pharmacologic treatment options available to help patients with obesity or metabolic syndrome to reduce body weight and to improve the glycaemic control [45]. The major adverse effects are gastrointestinal.

CSE showed capacity to reduce total cholesterol and triglycerides plasma levels in rats after 45 d treatment with CSE (2.2 mg caffeine/kg body weight and 0.8 mg CGA/ kg body weight). One mechanism of action seems to be the inhibition of pancreas lipase, a key enzyme for fat digestion, (Figure 5) since CSE reduced 41.73% its activity *in vitro* at concentration of 36 mg/mL (del Castillo et al. 2014). CGA (3.1 mg/mL) caused a 30.70% inhibition of enzyme activity while caffeine (1.56 mg/mL) was ineffective. All these results support the lyporegulatory character of CSE through the inhibition of pancreatic lipase and therefore its preventive and therapeutic effect in the obesity disease.

Several epidemiological investigations associate coffee consumption with the reduction ofan obesity risk. The anti-obesity effect of coffee may be ascribed to their bioactive compounds, caffeine, chlorogenic acids (CGAs) and melanoidins, which are present in coffee silverskin [6,8]. Different mechanisms have been proposed by CGA and caffeine regulate lipid metabolism. They can act by modulating cell signalling, reducing lipid accumulation and size of adipocytes [40], inhibiting pancreatic lipase [47], regulating hepatic lipid metabolism-related enzymes [48], and

by downregulating the genes involved in adipogenesis and inflammation in visceral adipose tissue [49] and transcription factors such as SREBP-1c and related molecules, which leads to the suppression of body fat accumulation [50]. In addition, coffee melanoidins have showed to protect against non-alcoholic fatty liver disease by reducing the hepatic fat accumulation in the rat model [51].

COFFEE SILVERSKIN EXTRACT AND DIABETES

The development of T2D is usually associated with a combination of insulin resistance and beta cell failure leading to high blood glucose levels. Insulin resistance is defined as a pathophysiological condition in which a normal insulin concentration does not adequately produce a normal insulin response in peripheral tissues, such as adipose, muscle and liver tissues [52]. Nevertheless, even undiagnosed patients are at increased risk of developing macrovascular and/or microvascular complications such as renal disease, retinopathy, arterial hypertension and its consequences, dyslipidaemias and obesity. Not all, but most patients with T2Dare overweight or obese. In fact, the excess weight itself causes some degree of insulin resistance. However, patients who are not obese or overweight but of traditional weight criteria may have a higher percentage of body fat predominantly distributed in the abdominal region [53,54].

Hyperglycaemia was estimated to be a major factor contributing to accelerated protein glycation and the formation of AGEs (AGEs) [55]. In diabetes, free radical formation by non-enzymatic glycation of proteins, glucose oxidation and increased lipid peroxidation, leads to damage of enzymes, cellular machinery and also increased insulin resistance [56]. In addition, oxidative stress is critically involved in the impairment of beta cell function due to their normal low antioxidant defence [57]. Oxidative stress and free radicals havea major role in the onset and progression of late diabetic complications such as coronary artery disease, neuropathy, nephropathy, and retinopathy [58]. *In vivo* studies support the role of hyperglycaemia in the enhancement of oxidative stress leading to endothelial dysfunction in blood vessels of diabetic patients [59].

The bioactive compounds present in CSE affect several pathways involved in the pathogenesis of diabetes, thereby reducing the risk of this disease. The effects of CSE on the biomarkers of diabetes can be summarised as follows:

1. Increased glucose tolerance [46]
2. Enhanced insulin sensitivity and secretion [46,60]
3. Inhibition of α-glucosidase activity [46]
4. Decreased total plasma cholesterol and triglycerides [46]
5. Inhibition of lipase activity [46]
6. Inhibition of AGEs formation through the interaction of CGA and its derivatives with protein backbone [61,62]
7. Enhanced antioxidant defence in beta cells against oxidative damage [46,60] causing reduction of oxidative stress and protein damage in diabetic pancreas [63]**.**

All these effects have an impact on diabetes and health (Figure 6). The components of CSE are metabolised and play a role with vital organs involved in the pathogenesis of diabetes and its complications. Consequently, CSE may be useful in both the prevention and treatment of diabetes.

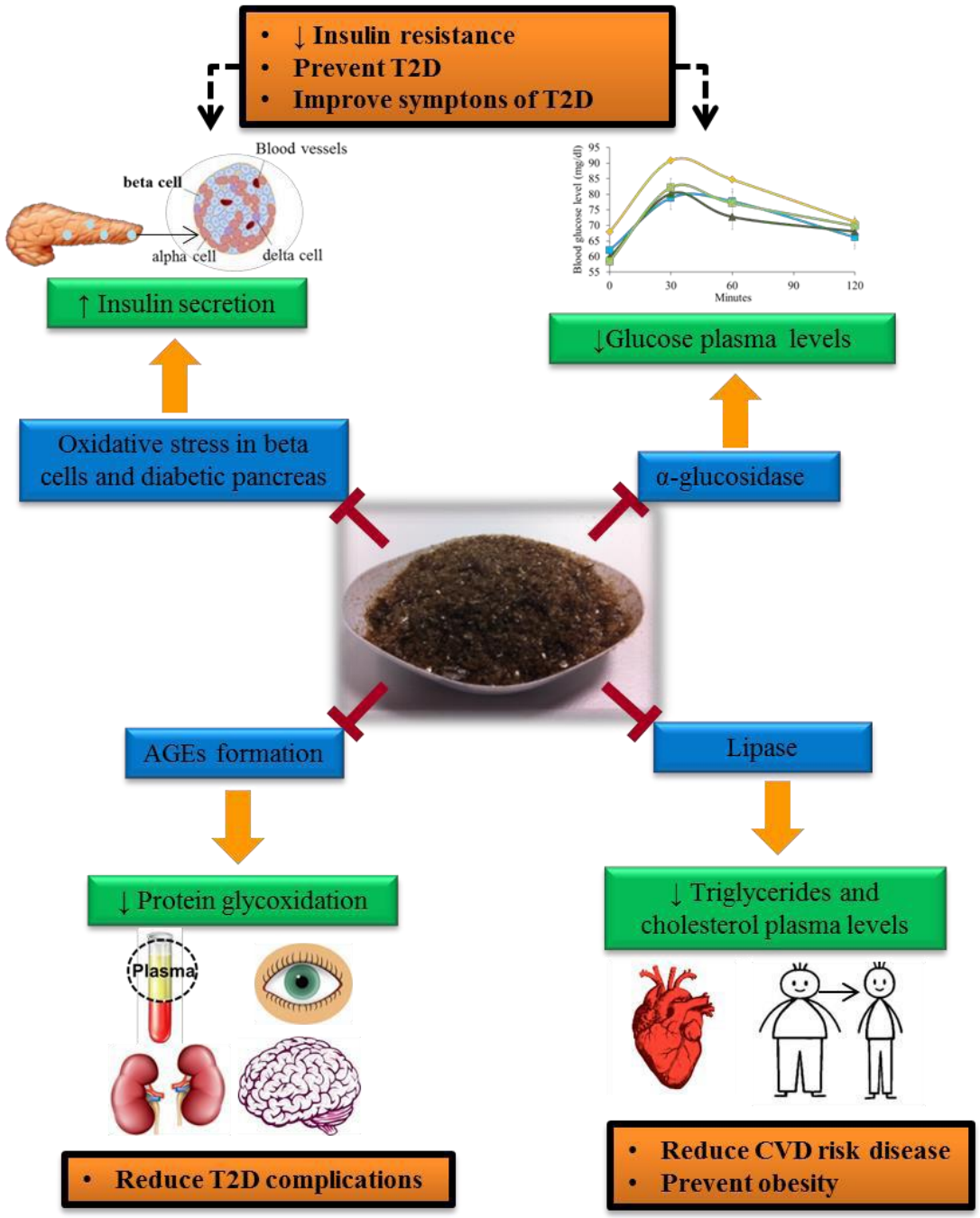


Figure 6. Effects of coffee silverskin extract on the biomarkers of Type 2 Diabetes and health.

Most studies on the effects of coffee components on glucose metabolism published to date are based on data obtained from animals and in vitro. Thus, the relevance of these results for the development of T2D in humans is currently unclear. However, these studies suggest that an effect of coffee consumption on glucose metabolism is biologically plausible, and that the effects of coffee cannot be equated to those of caffeine and CGA. Other coffee constituents relevant for diabetes are melanoidins, melatonin, lignans and lignin, tannic acid, isoflavones and trigonelline,

all of which act by following different pathways and may be present in CSE. Table 2 shows the effects of coffee components onT2D.

Caffeine concentrations in CSE range between 3-3.4 % (7). Our results show that caffeine in CSE was metabolized and the metabolites protected the pancreas against oxidative stress in rats suffering streptozotocin-induced diabetes [63]. Since no effect of caffeine was observed in INS-1E cells, results suggest that its metabolites are more effective than the parental molecule in preventing oxidative stress during diabetes. Caffeine can also reduce glucose levels and insulin sensitivity [64,65]. Caffeine treatment improved the health of the pancreas of diabetic rats. Other authors have also observed a protective effect of caffeine in pancreatic beta cells [66,67]. Food components should be bioavailable to exert their therapeutic effect. Our results support that caffeine and CGA are bioavailable and that they affect the biomarkers of diabetes [63]. Differences found between *in vitro* and *in vivo* studies highlight the interest of the study of the influence of molecule metabolism in the bioactivity of food components.

Table 2. Coffee components and possible effects in Type 2 Diabetes.

Component	Concentration in CSE (g per 100g)	Suggested mechanism
Caffeine	3-3.4	• Reduce glucose levels and insulin sensitivity • Protective effect against oxidative stress in beta cell and pancreas
CGA	1.1-6.8	• Regulate glucose metabolism • Enhance insulin action • Inhibit α-glucosidase activity • Protect beta cell and pancreas against oxidative stress • Inhibit AGEs formation
Melanoidins	17.2-23.9	• Antioxidant and antiglycative effects
Melatonin	0.34	• Protection of beta cells from oxidative stress
Isoflavones	nd	• Proliferation and protection of beta cells • Decrease HbA1c levels and improve lipid profile
Tannins	nd	• Hypoglycemic and antioxidant effects
Lignin	nd	• Decrease glucose absorption, improve insulin sensitivity and protect against oxidative stress
Lignans	nd	• Antioxidant action and decrease glucose, HbA1c, C-reactive protein and lipids plasma levels
Trigonelline	nd	• Improve insulin content and sensitivity • Regulate glucose and lipids metabolism

CSE, Coffee silverskin extract; nd, no data; HbA1c, glycated haemoglobin.

CGA is present in amounts between 1.1-6.8% in CSE (Table 1). Our results suggest that CGA and its metabolites have a greater effect on T2D biomarkers than caffeine. In previous studies, CGA and its roasting-formed derivates present in CSE were proposed as the main contributors to the beneficial effects of CSE on T2D [3,68,69]. The antidiabetic effect of CGA has been associated with different mechanisms, including: 1) regulation of glucose metabolism [3,70–73], 2) enhancement of insulin action [72–74], 3) inhibition of α-glucosidase activity [75,76], 4) protection against oxidative stress [14,16,77] and 5) inhibition of AGEs formation by different pathways (antioxidant properties, chelating properties, quenching of carbonyl radical

species and AGE crosslinking)[6,78]. Our results show that the formation of fluorescent AGEs is inhibited by different pathways such as carbonyl trapping, antioxidant effect and the formation of protein-phenols conjugates [6,61,62,78,79]. The finding of this new mechanism of action of CGA and CSE in the formation of AGEs is very relevant (Chapter 1) and was achieved by employing advanced analytical approaches also called phytochemomics [80]. Our study confirms the interest of the use of omics for a better understanding of the bioactivity of food components and, in particular, of those bioactive compounds present in low concentrations, such phenolic compounds. Most of the complications of diabetes are associated with AGEs [81]. In this sense, the search for natural sources of inhibitors of the formation of AGEs represents a scientific challenge.

Other compounds present in CSE are melanoidins. Melanoidin values between 17.2-23.9% have been found in the extract (Table 1). Antiglycative, chelating and antioxidant properties have been ascribed to coffee melanoidins [82–84]. These functions are linked to the presence of CGA, protein and polysaccharides in its complex structure [82]. Melanoidins from CSE have a carbonyl trapping capacity and inhibit fluorescent AGEs formation [6]. Hence, these compounds could be used as inhibitors of AGEs related diseases. Furthermore, melanoidins and CGA may contribute to a synergic inhibitory effect on the formation of AGEs. Further research should be conducted to elucidate the contribution of these individual compounds to this very important property in diabetes and its complications.

Melatonin is an indoleamine hormone [85,86]. Our CSE contains 3.4 mg/g dry matter of melatonin (Table 1 Experimental evidence indicated that melatonin has the potential to reduce the risk of T2D by protecting beta cells against oxidative stress, as it neutralizes the production of reactive species and normalizes the redox state in the cell [87]). Melatonin, CGA and other coffee antioxidants (CGA and its metabolites, caffeine metabolites, melanoidins among others) may exert synergic effects protecting the pancreas against oxidative stress and the development of diabetes. However, further research should be carried out in order to confirm this hypothesis.

Other compounds present in coffee and probably our CSE are isoflavones, tannic acid, lignin and lignans, and trigonelline. These compounds may also be responsible for the health-promoting properties associated with CSE. Although we have not analysed the presence of these molecules in our CSE, their identification would open a new field of study which would contribute to the knowledge of the effects of this extract on T2D.

Isoflavones are phenolic compounds described in coffee beans [88]. In processed coffee, these compounds are usually found as glucoside derivatives and free aglycones [89]. The most abundant isoflavones characterised in roasted coffee are genistein, daidzein and formononetin (methylated precursors of daidzein) beans [88]. Isoflavones levels in coffee beans (about 30-40%) decrease during the roasting process of the beans [88]. Genistein intake was associated with an antidiabetic effect through different mechanisms of action, such as direct effects on beta cell proliferation, glucose-stimulated insulin secretion and protection against apoptosis, apart from its functions as an estrogen receptor agonist, antioxidant or tyrosine kinase inhibitor [90]. Supplementation with genistein and daidzein caused a decrease in blood glucose and HbA1c levels and also improved the lipid profile in T2D animals [91]. Further research should be

conducted in order to find out the content of isoflavones in CSE and their contribution to the antidiabetic effects associated to the extract.

Tannins (commonly referred as tannic acid) are water-soluble polyphenols that are present in CS [92]. The amount of tannins reported in CS was 0.43 mg tannic acid equivalents/l [92]. The ingestion of tannic acid and other hydrolysable tannins has been related to anti-nutritional effects, since they form complexes with proteins, starch, and digestive enzymes causing a reduction in the nutritional value of foods. However, their antioxidant property is also well-documented and depends on the amount and type of tannins present in food [93]. The healthy effect of tannins might be related to other components associated with these molecules rather than to tannins themselves [93]. Tannins have been proposed as antidiabetic agents due to their hypoglycemic and antioxidant activities observed *in vitro* [94] and *in vivo* [95]. Therefore, tannins may contribute to the antiglycoxidative effect found for CSE.

CS also contains lignin, an organic polymer (28-30% dry matter), which is classified as insoluble dietary fibre [96]. Thus, lignin is resistant to digestion in the small intestine and requires colonic bacterial fermentation. An inverse relationship has been observed between the intake of insoluble fibre and the risk of developing T2D. Insoluble fibre may have a different mode of action in T2D, such as decreasing the absorption of simple carbohydrates and improving insulin sensitivity [97]. Metabolites of native lignin (lignophenols) have also been reported to reduce oxidative stress and inflammation in streptozotocin-induced diabetic rats [98]. Therefore, it would be interesting to study the content of lignans in CSE.

Other bioactive compounds related to lignin and present in coffee beans are lignans [99]. In plants, lignans (monolignol dimers) usually occur free (aglycone) or bound to sugars (glycoside). Monolignols, derived from hydroxycinnamic acids, are either dimerized to lignans into the cell or polymerized into larger lignin structures in the cell wall [100]. The enterolignans are metabolites of food lignans produced by human intestinal bacteria. They exert weak estrogenic [101] and other biochemical properties, suggesting a nutritional potential for preventing chronic diseases [102]. The main effects of lignans and their derivatives in the pathogenesis of T2D included the decrease of fasting glucose, HbA1c and C-reactive protein levels and the improvement of lipid profiles [90]. Lignans may affect glucose metabolism through the antioxidant and anti-estrogenic properties of their metabolites [90].

Trigonelline, a niacin related compound, is a natural constituent of coffee beans (approximately 1% dry matter) that is partially degraded to nicotinic acid during the roasting process [103]. Although we have not analysed the presence of trigonelline in our CSE, it is probably present since it is contained in roasted coffee beans. Trigonelline has beneficial effects on diabetes such as hypoglycaemic, hypocholesterolemic and hypotriglyceridemic effects. Previous studies have suggested that trigonelline may play a role in improving the insulin content in plasma and the pancreas, as well as the insulin sensitivity index in diabetic rats [104]. On the other hand, trigonelline regulates glucose and lipid metabolism through the inhibition of key enzymes [105]. Trigonelline may also contribute to the antidiabetic effect found for CSE. In agreement, the study of bioaccesibility, bioavailability and bioactivity of trigonelline is of great interest. Some of the effects ascribed to trigonelline in T2D correspond to those found for CSE. Trigonelline may also contribute to the antidiabetic effect found for CSE. Thus, the study of the

bioaccesibility, bioavailability and bioactivity of trigonelline is of great interest. Some of the effects ascribed to trigonelline in T2D correspond to those found for CSE.

SUMMARY

- The coffee by-product generated during roasting is named coffee silverskin and presents a complex chemical composition.
- CSE contains coffee components able to reduce the risk of accelerated aging and chronic metabolic disorders.
- The contribution of the individual bioactive compounds responsible for the health promoting effects of CSE should be elucidated. Other compounds besides CGA and caffeine seem to play a role on the biomarkers of chronic diseases. These effects may be associated to its antioxidant power and capacity to inhibit enzymes involved in the metabolism of nutrients (carbohydrates and lipids).
- The valorisation of CS into food supplements or functional food ingredients for chronic diseases is feasible. Further experiments should be performed to demonstrate the health promoting properties of CSE in humans.
- The use of CS for chronic diseases makes coffee production more sustainable.

TEST QUESTIONS

1. What is coffee silverskin?

a. The roasting coffee by-product
b. Coffee fruit
c. A new commercial functional beverage
d. The skin of the coffee cherry, the outset layer

2. Which of the following answers is NOT one of the main causes of chronic diseases such as diabetes, obesity and the metabolic syndrome?

a. Aging
b. Pathogen infection
c. Oxidative stress
d. Inflammation

3. Why has *C. elegans* been widely used in aging studies? Because…

a. The genome for *C. elegans* has been completely sequenced
b. It has simple growth conditions and reproduces rapidly with a short life span
c. It has evolutionarily conserved pathways for aging
d. All of the above

4. Which compounds present in the coffee silverskin extract have shown to reduce fat accumulation in *C. elegans*?

a. Chlorogenic acid (CGA) and proteins
b. CGA and melanoidins

c. **CGA and caffeine**
d. CGA and fibre

5. Which enzymes involved in the human digestion process can be inhibited by the consumption of coffee silverskin extract?

a. Lipase and α-amylase
b. Lipase and pepsin
c. Lipase and lactase
d. **Lipase and α-glucosidase**

6. Which effects showed the consumption of coffee silverskin extract on the biomarkers of diabetes?

a. Increased glucose tolerance , reduced insulin sensitivity and enhanced AGEs formation
b. Reduced glucose tolerance, increased insulin sensitivity and enhanced AGEs formation
c. **Increased glucose tolerance, increased insulin sensitivity and inhibited AGEs formation**
d. Increased glucose tolerance, increased insulin sensitivity and enhanced AGEs formation

7. Which compounds in the coffee silverskin extract besides chlorogenic acid and caffeine may help to reduce the risk of type 2 diabetes?

a. Melatonin and trigonelline
b. Melatonin and melanoidins
c. Melatonin and isoflavones
d. Melatonin and tannic acid

8. Why the valorization of coffee silverskin in functional products for humans is interesting?

a. It is not interesting at all
b. **It is a good strategy for the sustainability of both coffee production and human health**
c. You can make nice colorful cosmetics and lot of money
d. Use it for compost.

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List of Abbreviations: Advanced glycated end products, AGEs; Chlorogenic acid, CGA; Coffee silverskin extract, CSE; Coffee silverskin, CS; Deoxyribonucleic acid, DNA; Food and Drug Administration, FDA; Free fatty acids, FFA; Glutathione peroxidase, GPx; Glutathione, GSH;

Low density lipoprotein, LDL; Reactive oxygen species, ROS; Type 2 diabetes, T2D; Ultraviolet C; World Health Organization, WHO

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